SYNOPSIS OF RESEARCH PROJECT

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4.	**Name of the Degree Submitted for**	:	Ph. D. (Agri.)
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8.	**Title of Research Project**	:	**EFFECT OF FOLIAR APPLICATION OF ZINC SULPHATE AND ZINC NANOPARTICLES ON PHYSIO-BIOCHEMICAL PARAMETERS AND ENZYME ACTIVITIES UNDER SALINITY STRESS IN PEARL MILLET [*PENNISETUM GLAUCUM* (L.) R. BR.]**

9. Introduction

Millets have long been a traditional staple food in the dryland regions of the world. Their cultivation dates back to around 4500 BC in both China and India. Archaeological evidence from the Neolithic site of Sanganakallu in Ballari, Karnataka, indicates that millets were cultivated and processed as early as 3000 BC, nearly 5000 years ago (Soumya *et al*., 2023).

Millets are often described as “yesterday’s coarse grains and today’s nutri-cereals.” They are increasingly recognized as “future crops” due to their resilience against pests and diseases and their ability to thrive in the harsh conditions of arid and semi-arid regions across Asia and Africa. Millets are small-seeded grains, with the most important

food varieties including sorghum (*Sorghum bicolor* L.), pearl millet (*Pennisetum glaucum* L.), finger millet (*Eleusine coracana* L.), teff (*Eragrostis tef* L.), proso millet (*Panicum miliaceum* L.), kodo millet (*Paspalum scrobiculatum* L.), foxtail millet (*Setaria italica* L.), little millet (*Panicum sumatrense* L.) and fonio (*Digitaria exilis* L.), (Gowda *et al.*, 2022).

India made proposal to designate of 2023 as the "International Year of Millets," building on its earlier declaration of 2018 as the "National Year of Millets." The country is the world's largest producer of millets, followed by Nigeria and China. In India, millets are cultivated on approximately 17 million hectares, with an annual production of about 18 million tonnes, contributing nearly 10% to the national food grain basket. The major millets grown in India include sorghum (*Sorghum bicolor* L.), pearl millet (*Pennisetum glaucum* L.) and finger millet (*Eleusine coracana* L.), (Soumya *et al*., 2023).

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a diploid species with chromosome number of 2n = 2x = 14 considered as an important tropical C_4 small-grained cereal belonging to the family Poaceae and subfamily Panicoideae. It is a highly tillering, cross-pollinated crop with a short life cycle and a relatively large genome size (~2352 Mbp) (Bennett *et al*., 2000). Pearl millet is predominantly cultivated in the marginal environments of arid and semi-arid regions of Sub-Saharan Africa and the Indian subcontinent, where conditions such as low and erratic rainfall, poor soil fertility and high temperatures limit the growth of staple cereals like rice, wheat, maize and even sorghum (Vadez *et al*., 2012).

It ranks as the sixth most economically important cereal crop and plays a crucial role in ensuring food security for nearly 90 million people living in these regions (Agarwal *et al*., 2010). As a dryland crop, it is highly tolerant to environmental stresses, including drought, heat, and nutrient-deficient soils, where other cereals often fail. Although primarily grown for grain production, pearl millet is also utilized by marginal farmers for fodder, hay, bird feed and fuel. Globally, it is cultivated on about 31 million hectares, contributing nearly 50% of total millet production. In India, it occupies around 7.8 million hectares with an annual grain production of approximately 9.25 million tonnes (Ali *et al*., 2004).

Pearl millet is a highly nutritious cereal that plays a significant role in the human diet due to its rich content of calcium, iron, zinc, lipids and high-quality proteins. Carbohydrates form the largest portion of the grain accounting for about 71.82 to 81.02% (Cheik *et al*., 2006). Compared to sorghum and other millets, pearl millet generally contains higher levels of protein and fat largely because its grain is a naked caryopsis. Its protein is

not only abundant but also of relatively good quality though it is limited in lysine. The protein and fat contents in different pearl millet varieties range from 12.25 to 13.09% and 4.32 to 5.11%, respectively (Abdalla *et al*., 2009), while protein digestibility varies between 47.30 and 61.17% (Anju, 2005). Total sugar content ranges from 2.55 to 2.93%, including 2.15 to 2.57% non-reducing sugars and 0.34 to 0.39% reducing sugars (Rekha, 1997). In addition, pearl millet has a superior amino acid and fatty acid composition compared to many other cereals. The grain also contains notable levels of anti-nutritional factors, such as phytic acid (603.33–678.33 mg/100 g) and polyphenols (502.78–658.30 mg/100 g) (Anju, 2005). Furthermore, it provides 4.31–5.30 percent crude fibre, 1.53–2.00 percent ash and important minerals including phosphorus (450–990 mg), calcium (10–80 mg), iron (7–18.0 mg), zinc (5.3–7.0 mg), copper (1.0–1.8 mg) and manganese (1.8–2.3 mg) per 100 g of grain (Abdalla *et al*., 2009). Hundred grams of bajra grain contains- energy 360 calories, moisture 12 g, protein 12 g, fat 5 g, mineral 2 g, fiber 1 g, carbohydrate 67 g, Calcium 42 mg, phosphorus 242 mg and iron 8 mg.

During 2024-25, pearl millet was cultivated over about 6.83 million hectares in India, with an average productivity of 1,488 kg per hectare, resulting in an estimated total production of 9.49 million tonnes, according to the second advance estimates of the Department of Agriculture & Farmers Welfare.

The crop is predominantly grown in a few key states—Rajasthan, Maharashtra, Uttar Pradesh, Gujarat, and Haryana—which together account for around 90% of the country's total output. Rajasthan is the leading producer, contributing nearly 45% of production, followed by Uttar Pradesh 19%, Haryana 10%, Gujarat 9%, Madhya Pradesh 7%, Maharashtra 6%, Karnataka 3% and Tamil Nadu 2%.

Pearl millet is mainly cultivated during the rainy season, typically from June or July to September or October. It is also grown during the summer season (February to May) in certain regions of Gujarat, Rajasthan and Uttar Pradesh. Additionally, limited cultivation takes place in the post-rainy (*rabi*) season (November to February), particularly in parts of Maharashtra and Gujarat (ICAR pearl millet project, 2025).

Soil salinity is increasingly recognized as a major environmental constraint, affecting more than 20% of the world's irrigated land and significantly reducing the availability of arable land (Hussain *et al*., 2016). Salinity interferes with key metabolic processes in plants, inducing oxidative, ionic and osmotic stress that collectively impair growth and physiological functioning (Munns and Tester, 2008). These stresses lead to a range of physiological disorders including reduced stomatal conductance, decreased

photosynthetic efficiency, nutrient imbalance and increased production of reactive oxygen species along with lipid peroxidation. As a result, plant growth is adversely affected leading to reductions in biomass, root length and overall plant vigour. (Noor *et al*., 2022).

To meet the United Nations' Zero Hunger target by 2030, there is a pressing need to rethink and improve conventional agricultural practices. Traditional breeding approaches, such as interspecific and intergeneric hybridization have achieved limited success in enhancing stress tolerance in crops. In recent years nanoparticles (NPs) have emerged as a promising tool for improving plant tolerance to salinity stress and supporting sustainable agriculture (Ahmad and Akhtar, 2019). Evidence from multiple studies suggests that NPs can significantly enhance plant growth at various developmental stages by modulating morphological, physiological and biochemical processes thereby increasing salinity tolerance (Etesami *et al*., 2021). It is hypothesized that nanoparticles contribute to stress tolerance by regulating hormone levels, maintaining ionic homeostasis, enhancing antioxidant enzyme activity and influencing gene expression and plant defense mechanisms (Wahid *et al*., 2020).

Zinc deficiency in agricultural soils is a major constraint affecting both crop productivity and the nutritional quality of millets grown in semi-arid regions. These crops are typically cultivated under conditions of low rainfall and are vital in economically vulnerable areas where food security is challenged by recurrent droughts and poor soil fertility (Dimkpa *et al*., 2019). In India, the widespread deficiency of zinc in soils is largely due to intensive cultivation of cereals and legumes without adequate crop rotation or fallow periods. This continuous cropping practice leads to soil nutrient depletion, ultimately resulting in reduced yields and lower mineral content in the produce. (De Groote *et al*. 2021).

Zinc plays a crucial role in addressing nutritional deficiencies and malnutrition prevalent in many developing countries including India, where it significantly affects children's health (Dubey *et al*., 2025). Although pearl millet is a hardy crop, it is often cultivated in zinc-deficient soils making this micronutrient deficiency a common concern. Zinc deficiency not only reduces crop productivity and the nutritional quality of grains but also poses serious health risks when dietary intake is insufficient (Tiwari and Yadav, 2020). In plants, zinc is essential for numerous physiological and biochemical processes, including protein synthesis, photosynthesis, enzyme activation, membrane stabilization and the metabolism of lipids, proteins and carbohydrates (De La Torre-Roche *et al*., 2020).

Nanoparticles have emerged as an efficient means of delivering essential nutrients to plants, improving their absorption and utilization while minimizing nutrient losses and enhancing overall agricultural productivity. In addition to their role in nutrient delivery, nanoparticles can also promote plant growth and development (Manzoor *et al*., 2024). Furthermore, they hold significant potential for strengthening crop resilience within sustainable agricultural systems by reducing reliance on conventional chemical inputs and enabling plants to better adapt to changing climatic conditions and environmental stresses (Preetha *et al*., 2023).

Zinc oxide nanoparticles (ZnO-NPs) have gained considerable attention due to their advantages over conventional zinc fertilizers, including higher bioavailability, controlled nutrient release and a large surface area. These nanoparticles can interact with plant cell walls to form nanoscale apertures, facilitating their entry and movement through both apoplastic and symplastic pathways (Schwab *et al*., 2016). Among metal oxide nanoparticles, ZnO-NPs are particularly important and widely used across various sectors because of their unique physicochemical properties (Smijs and Pavel, 2011). ZnO-NPs provide a more soluble and readily available form of zinc to plants, helping to overcome zinc deficiency, which is often caused by the low solubility of zinc compounds in soil. Foliar application of ZnO-NPs is considered especially effective, as it enables rapid nutrient uptake through leaf pores or stomata, bypassing root limitations and enhancing photosynthesis, enzyme activity and grain development (Tarafdar *et al*., 2014). Several studies have shown that foliar application of ZnO-NPs can significantly improve grain yield, zinc accumulation and overall growth performance in millets.

Therefore, the present study aims to evaluate the effect of $ZnSO_4$ and ZnO nanoparticles as a foliar supplement in pearl millet cultivation with a particular focus on their impact on yield, nutritional quality and plant performance under zinc-deficient conditions in a controlled environment.

10. Practical Utility of the Research Project

The study on the effect of foliar application of Zinc sulphate and Zinc Oxide Nanoparticles (ZnO-NPs) on biochemical parameters and enzyme activities under salinity stress in pearl millet has important practical applications in agriculture. Salinity is a major constraint that reduces crop growth and yield in many arid and semi-arid regions. The application of ZnO-NPs can improve the tolerance of pearl millet plants to salt stress by enhancing physiological performance, biochemical activities and antioxidant defence mechanisms. This helps plants to maintain better growth, photosynthesis and metabolic

balance even under adverse conditions. The use of $ZnSO_4$ and ZnO-NPs can also improve nutrient uptake efficiency and may increase zinc content in grains, thereby enhancing the nutritional quality of pearl millet. Furthermore, the findings of this research can contribute to the development of nano-fertilizers that require lower doses compared to conventional fertilizers and reduce environmental pollution. Therefore, the study provides a scientific basis for developing sustainable crop management strategies and improving productivity of pearl millet in saline and marginal soils.

11. Objectives

1. To synthesise and characterize Zinc Oxide Nanoparticles.
2. To evalute the effect of $ZnSO_4$ and ZnO-NPs on physiological and biochemical parameters in pearl millet plant under salt stress.
3. To evalute the changes in antioxidant enzyme and metabolite influenced by foliar application of $ZnSO_4$ and chemically synthesized ZnO-NPs in pearl millet.
4. To study the response of $ZnSO_4$ and ZnO-NPs on yield and yield attributes.

12. Review of Literature

The present review is an attempt to bring together some of the findings on physiological, biochemical parameters, antioxidant enzyme activity and metabolite study of pearl millet [*Pennisetum glaucum* (L.) R. Br.]

12.1 Synthesis and characterization of ZnO-NPs

El-Waseif *et al*. (2017) reported that for the chemical synthesis of zinc nanoparticles, 0.1 M of $ZnSO_4 \cdot 7H_2O$ solution was added to 0.2 M NaOH solution. The mixture was placed in a beaker on a hot plate at 55 °C and stirred at 750 rpm. The solution was stirred for about 20 minutes. The appearance of a white milky solution indicated the formation of zinc nanoparticles (Zn-NPs).

According to Kolenčík *et al*. (2019), zinc oxide nanoparticles for foliar application were obtained from Sigma-Aldrich (Saint Louis, MO, USA). The commercial code 721077 ZnO nanoparticles with an average particle size of ≤40 nm and 20 wt.% concentration were diluted in H_2O and subsequently analysed by X-ray diffraction (XRD) using a Bruker D8 DISCOVER diffractometer (Bruker, MA, USA). A 12 kW (40 kV, 300 mA) Cu-anode was employed for the XRD measurements and unit cell parameters were calculated using TOPAS 3.0 (Bruker, MA, USA). The dimension range and morphology of the nanoparticles were visualized using JEOL 7610F+ scanning electron microscope, while the chemical composition of the qualitative components was verified using an energy-dispersive X-ray spectrometer (EDAX) attached to a Philips XL30.

Nandhini *et al*. (2019) synthesized ZnO nanoparticles by adding 10 mL of 50 kDa retentate of aqueous *E. alba* extract to 80 mL of zinc sulphate solution (10 mM) and the mixture was stirred for 48 h. The solution was then stirred vigorously at 100 °C for 2 h. Afterward, the solution was centrifuged and the supernatant was discarded. The residue was poured onto a plate and dried at 60 °C for 7–8 h. The white solid product was scraped off the plate and stored for further experiments.

Kumar *et al*. (2024) made study on zinc oxide nanoparticles (ZnO NPs) and synthesized using zinc nitrate and *Calotropis procera* leaf extract and characterized using techniques such as UV–visible spectroscopy, EDX, SEM, FTIR and XRD. Seeds of the hybrid pearl millet "HHB 67 Improved" were primed with ZnO NPs, $ZnSO_4$ and hydropriming to compare their effects. The results showed that 150 ppm ZnO NPs increased seed germination by 20% and the vigor index by 51% under laboratory conditions.

Gupta *et al*. (2024) used transmission electron microscopy (TEM), X-ray diffraction (XRD), and selected area electron diffraction (SAED) to determine the morphology and actual size of ZnO nanoparticles. The XRD planes corresponding to the (002), (101), (102) and (202) planes indicated typical zinc oxide crystals. The hexagonal rod-shaped ZnO nanoparticles had an average length of 87.52 nm and a width of 39.76 nm.

In the study conducted by Mustafa *et al*. (2024), ZnO nanoparticles were synthesized as a colloidal suspension using a sol–gel technique. Briefly, 0.5 mol L^{-1} (8.78 g) of zinc acetate dihydrate (99.5%) was dissolved in 80 mL of 2-propanol (99.9%) at 50°C. The solution was then diluted to 840 mL with 2-propanol. Subsequently, 160 mL of a 0.5 mol L^{-1} sodium hydroxide solution (3.2 g in 160 mL), prepared using a 7:1 mixture of 2-propanol and deionized water was added at 0°C within 1 min under continuous stirring. The mixture was immersed in a water bath preheated to 65 °C for 2 h. After aging for seven days at room temperature the solvent was removed by centrifugation at 7000 rpm for 15 min. The centrifugation process was repeated until the by-products were completely removed. The precipitate was then dried in an oven at 120 °C for 8 h. Finally, the powder was calcined in a muffle furnace at 400 °C for 2 h followed by further calcination at 500°C for 2 h to obtain ZnO nanoparticles.

12.2 Physiological parameters

Satish *et al*. (2016) reported that salinity stress significantly influences the physiological performance of finger millet genotype 'CO(Ra)-14'. Increasing NaCl concentrations reduced shoot and leaf growth, while root length increased, indicating an

adaptive response to stress. Membrane damage was evident from increased electrolyte leakage and elevated proline content suggested its role in osmotic adjustment.

Yavas and Unay (2016) reported that zinc (Zn) application plays an important role in alleviating the adverse effects of drought stress in wheat. Drought conditions negatively affected growth, yield attributes, chlorophyll content and relative water content; however, foliar application of Zn significantly improved these parameters. Zinc enhanced water use efficiency helping plants maintain a better physiological status under stress conditions. Overall, Zn application proved effective in mitigating drought-induced damage and improving wheat growth and performance.

Dimkpa *et al*. (2019) observed that zinc oxide nanoparticles improved plant tolerance to salinity by regulating ion transport and maintaining a favorable Na^+/K^+ ratio in plant tissues. This regulation helped maintain cellular osmotic balance and protected plants from salt-induced stress.

Sharma *et al*. (2021) reported that rice seeds treated with ZnO nanoparticles exhibited increased radicle length, higher relative water uptake and greater seedling dry weight compared to hydro-primed controls.

Kumar *et al*. (2024) noticed that ZnO nanoparticles treatment improved agronomic traits, including plant height, leaf area, chlorophyll content, fresh weight and dry weight in pearl millet. The treated plants also exhibited strong antioxidant activity with 78.11% DPPH radical scavenging, which was higher than that observed in $ZnSO_4$-treated and control plants.

Mushtaq *et al*. (2023) observed that low doses of exogenous zinc were effective in mitigating the adverse effects of NaCl by improving morphological and biochemical traits in proso millet. In salt-treated plants zinc at 1 mg/L and 2 mg/L alleviated the negative impact of 150 mM NaCl, resulting in increases in shoot length (SL) by 7.26% and 25.5% root length (RL) by 21.84% and 39.07% and membrane stability index (MSI) by 132.57% and 151.58% respectively.

Saleem *et al*. (2023) examined the mitigating role of zinc (Zn) in salt (NaCl)-stressed foxtail millet by focusing on the modulation of the ascorbate–glutathione (AsA–GSH) pathway. The individual and combined effects of Zn (1–5 mg L^{-1} Hoagland solution) and NaCl (150 and 200 mM) were evaluated on the morpho-physiological and biochemical parameters of *Setaria italica*. Salt stress significantly reduced plant growth, photosynthetic pigments, membrane stability index (MSI) and relative water content (RWC).

Gupta *et al*. (2024) reported that salinity stress significantly reduced relative water content (RWC) in leaves by 27% compared with control plants. However, the application of ZnO nanoparticles improved RWC under salt stress with foliar application increasing RWC by 50–78%, nano-priming by 40–75% and soil application by 27–41% compared with salt-stressed plants.

Mustafa *et al*. (2024) investigated the combined effects of ZnO nanoparticles and salinity, observing that different concentrations produced varying outcomes. Notably, only 50 mM NaCl combined with 50 ppm ZnO NPs showed positive effects on pea physiology, resulting in a substantial increase in root length and improvements in other physiological parameters.

Pandya *et al*. (2024) conducted an investigation to study the effects of different concentrations of ZnO nanoparticles (ZnO NPs) on the germination and physio-biochemical parameters of wheat. Seeds were primed with ZnO NPs at concentrations of 5, 50, 100, 250 and 500 ppm for 4 hours with continuous aeration. The primed seeds were then grown for 21 days and evaluated. The results showed that 250 ppm ZnO NPs achieved 100% germination and significantly improved shoot length, root length and the fresh and dry weights of leaves and roots compared to the control. However, at 500 ppm, these growth parameters declined, indicating that 250 ppm was the optimal concentration for enhancing wheat growth.

12.3 Biochemical parameters

Abou-Zeid *et al*. (2021) reported that under salt stress, wheat plants primed with ZnO nanoparticles exhibited higher levels of chloroplastic pigments.

Mahadik and Kumudini, (2020) reported Proline accumulates at higher rates in salt-tolerant cultivars than in salt-sensitive finger millet plant.

Rai-Kalal and Jajoo (2021) reported that ZnO nanopriming significantly enhanced photosynthetic pigments including chlorophyll a, chlorophyll b and total chlorophyll content in wheat.

Singh *et al*. (2022) reported that proline accumulation was reduced in rice leaves treated with ZnO nanoparticles, indicating a physiological adaptation to saline conditions. In addition, ZnO-NP-treated genotypes showed improved stomatal and trichome morphology, restored photosynthetic pigment accumulation and enhanced antioxidant enzyme activity, which collectively reduced oxidative stress.

In pearl millet, Kumar *et al*. (2024) found that treatment with 150 ppm ZnO nanoparticles resulted in the greatest increases in chlorophyll a and b (12.13% and 11.22%,

respectively) compared with water-treated controls. Plants treated with $ZnSO_4$ also showed increases in chlorophyll a and b (9.1% and 7.9%, respectively), but to a lesser extent than ZnO-NPs.

Pandya *et al*. (2024) treated wheat seeds with ZnO nanoparticles at concentrations of 5, 50, 100, 250 and 500 ppm for 4 hours with continuous aeration. The primed seeds were then sown in plastic bags and grown for 21 days. The results showed that ZnO nanoparticles significantly improved biochemical parameters, including increased total chlorophyll, malondialdehyde (MDA), total protein and the accumulation of osmolytes such as proline and glycine-betaine.

Prajapati *et al*. (2025) investigated the dose-dependent effects of foliar-applied ZnO nanoparticles on pearl millet (*Pennisetum glaucum*), testing concentrations of 0.001, 0.01, 0.1, 1, 2 and 10 ppm. Chlorophyll levels increased with ZnO-NP application up to 2 ppm, indicating a stimulatory effect on photosynthetic pigment accumulation, growth, antioxidant enzyme activities and yield traits.

Shivhare *et al*. (2025) studied the effects of zinc oxide nanoparticles (ZnO NPs) applied at different concentrations (0, 50, 100, 150, 200 and 500 mg kg^{-1}) on *Pennisetum glaucum* over a 30-day period. Moderate concentrations (100 and 150 mg kg^{-1}) slightly increased alpha-amylase activity, shoot and root length, biomass and photosynthetic pigments. Notably, plants treated with 150 mg kg^{-1} ZnO NPs showed a significant increase in total chlorophyll content compared to the control with an increase of approximately 45.33% after 10 days and 47.75% after 30 days.

12.4 Antioxidant enzymes

Upadhyaya and Roy (2017) reported that Zn NP treatment of the rice cultivar *Kmj-6-1-1* (Karimganj, Assam, India) at concentrations of 0–50 mg/L significantly enhanced radicle and plumule length, fresh and dry mass and seed moisture content. Additionally, antioxidant enzymes such as guaiacol peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD) and glutathione reductase (GR) were elevated, suggesting that Zn NPs protect seeds from ROS damage during germination. Consequently, Zn NP-treated seeds exhibited improved germination potential. The study emphasizes the need for further genomic analyses to elucidate the molecular mechanisms underlying Zn NP-mediated modulation of rice seed germination.

Adrees *et al*. (2021) and Hussain *et al*. (2021) Conducted field experiment to evaluate the effects of individual and combined applications of ZnO, Fe and Si nanoparticles (NPs) on wheat growth and cadmium (Cd) uptake under Cd-contaminated

conditions. The nanoparticles were applied as foliar sprays, both individually and in all possible combinations. Additionally, chlorophyll and carotenoid contents, as well as antioxidant enzyme activities were enhanced, while electrolyte leakage was reduced in NP-treated plants. Overall, the application of nanoparticles improved plant growth and reduced stress effects under Cd contamination.

Singh *et al*. (2022) highlighted the potential role of zinc oxide nanoparticles (ZnO-NPs) in enhancing plant growth and stress tolerance. In rice foliar application of ZnO-NPs at concentrations ranging from 0.001 to 10 ppm significantly affected antioxidant enzyme activities. Moderate concentrations, particularly 2 ppm enhanced the activities of catalase, peroxidase, superoxide dismutase (SOD) and ascorbate peroxidase (APX) indicating improved antioxidative defense, whereas higher concentrations reduced enzyme activity, likely due to toxicity.

Basit *et al*. (2022) reported that rice plants defend against stress by increasing the synthesis of antioxidant enzymes and phytohormones while reducing ROS formation, thereby preventing or minimizing physiological damage.

Saleem *et al*. (2023) examined the mitigating role of zinc (Zn) in salt (NaCl)-stressed foxtail millet by focusing on the modulation of the ascorbate–glutathione (AsA–GSH) pathway. The individual and combined effects of Zn (1–5 mg L^{-1} Hoagland solution) and NaCl (150 and 200 mM) were evaluated on the morpho-physiological and biochemical parameters of *Setaria italica*. Electrolyte leakage (EL), secondary metabolite content and antioxidant enzyme activities increased with increasing salinity. Lower doses of Zn (1–3 mg L^{-1}) alleviated the negative effects of salinity by improving the morpho-physiological and biochemical parameters of *S. italica*. With Zn supplementation enzymatic antioxidant components—superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR) and glutathione reductase (GR)—showed maximum increases.

Prajapati *et al*. (2025) investigated the dose-dependent effects of ZnO nanoparticles (ZnO-NPs) on the growth, antioxidative enzyme activities and yield traits of pearl millet (*Pennisetum glaucum*). Plants were treated with graded concentrations of ZnO-NPs (0.001, 0.01, 0.1, 1, 2 and 10 ppm) through foliar application and their effects were compared. Catalase and peroxidase activities showed notable variations in response to different ZnO-NP dosages. Catalase activity exhibited an upward trend reaching the highest H_2O_2 decomposition at 2 ppm.

Shivhare *et al*. (2025) studied the effects of zinc oxide nanoparticles (ZnO NPs) applied at concentrations of 0, 50, 100, 150, 200 and 500 mg kg^{-1} on *Pennisetum glaucum* over a 30-day period. Antioxidant activity significantly increased at 100 and 150 mg kg^{-1}, while higher concentrations (200 and 500 mg kg^{-1}) showed a decline, indicating negative effects at elevated levels. The highest free radical scavenging activity was observed at 100 and 150 mg kg^{-1} with DPPH inhibition reaching 31.39% and 42.61% after 10 days, 48% and 55.65% after 20 days and 54.69% and 55.78% after 30 days, respectively.

12.5 Yield attributes

Tarafdar *et al*. (2014) reported that nano-fertilizers enhanced nutrient use efficiency and improved crop productivity in pearl millet. The study highlighted that nano-zinc fertilizers provided a slow and controlled release of nutrients, thereby reducing nutrient losses and improving plant growth.

Siddiqui *et al*. (2015) observed that ZnO nanoparticles improved germination percentage, seedling vigor and early plant growth in several crops. The study concluded that nanoparticle-based fertilizers could be a promising approach for enhancing crop productivity.

Rameshraddy *et al*. (2017) investigated the effect of nano-zinc fertilizers on pearl millet and reported significant improvement in growth parameters such as plant height, number of tillers and dry matter production compared with conventional zinc fertilizers.

Solanki and Laura (2018) reported that zinc deficiency is a major nutritional problem in developing countries like India. Zinc fortification of wheat is therefore a research priority. Treating wheat seeds with nanoscale ZnO, bulk $ZnSO_4$ and ZnO suspensions improved seed germination, seedling vigor, root and shoot growth, biomass and chlorophyll content with nanoparticles showing the most pronounced effects on early plant development.

Itroutwar *et al*. (2020) found that priming maize seeds with ZnO nanoparticles (ZnO NPs) enhanced germination percentage and seedling parameters—including leaf length (60 mm), shoot width (3.4 mm), shoot length (13.0 cm), root width (1.0 mm), root length (20.7 cm), dry matter production (5.33 g) and vigor index (2931.9)—more effectively than zinc acetate treatment or hydro-primed controls.

Gupta *et al*. (2024) observed that salinity stress significantly reduced the growth of bean plants, decreasing fresh weight by approximately 24% compared with control plants. However, the application of zinc oxide nanoparticles (ZnO NPs) markedly improved plant fresh weight under saline conditions. Fresh weight increased by 39–83% through

foliar application, 60–68% via nano-priming, and 31–55% through soil application compared with salt-stressed plants.

Sumersinh *et al*. (2024) showed that seed priming with 800 ppm nano ZnO for 2 hours combined with foliar application of nano ZnO at 600 ppm produced the highest pearl millet yield (2077 kg/ha grain, 5569 kg/ha stover and 7646 kg/ha biological yield). This treatment performed similarly to foliar application at 500 ppm. The increased yield is attributed to efficient absorption and translocation of ZnO nanoparticles in plant cells, which enhanced growth traits such as effective tillers per plant, ear head length and overall grain and stover yield.

Prajapati *et al*. (2025) assessed the dose-dependent effects of ZnO nanoparticles (ZnO-NPs) on the growth, antioxidative enzyme activities and yield traits of pearl millet (*Pennisetum glaucum*). Plants were treated with graded concentrations of ZnO-NPs (0.001, 0.01, 0.1, 1, 2 and 10 ppm) through foliar application and their effects were compared. A marginal enhancement in seed production (0.248 g plant^{-1}) was observed at 0.1 ppm. Treatment with 1 ppm ZnO-NPs notably increased tiller number (7) and seed weight (1.648 g plant^{-1}). Maximum yield parameters—including tiller number (10), panicle length (10.8 cm), panicle weight (4.73 g) and seed weight (3.697 g plant^{-1})—were achieved at the 2 ppm ZnO-NPs treatment.

13. Materials and Methods

1	**Title of experiment**	Effect of foliar application of zinc sulphate and zinc nanoparticles on physio-biochemical parameters and enzyme activities under salinity stress in pearl millet [*Pennisetum glaucum* (l.) R. Br.]
2	**Location**	Department of Biochemistry, College of Agriculture, JAU, Junagadh.
3	**Year and season of experiment**	2026, *Kharif*
Experiment 1: Screening of pearl millet varieties during *in vitro* germination under saline water.		
1	**Crop and varieties**	Pearl millet
2	**Number of salt treatments**	T_0: Distilled water T_1: 2 EC saline water T_2: 4 EC saline water T_3: 6 EC saline water

		T_4: 8 EC saline water (Source: Saline water will be prepared by using NaCl or sea water).
3	**Design**	FCRD (Factorial Complete Randomized Design) 1st Factor: Variety:10 2nd Factor: Saline water treatments: 5
4	**No. of Replications**	Three
5	**Sampling**	Pearl millet seedlings will be collected at 10 DAS for physiological and biochemical analysis
6	**Observations to be recorded**	
6A	**Physiological parameters**	
1	**Germinations percentage (%)**	*In vitro* germination percentage will be done from pearl millet seed germinated in petri plate at 10 DAS (I. S. T. A., 1976).
2	**Shoot length in cm**	Shoot length and root length will be measured using ruler in terms of cm, five plants will be selected for measurement of shoot length and root length (Marcela *et al*., 2014).
3	**Root length in cm**	
4	**Seedling length in cm**	The total length, encompassing the distance from the root tip to the shoot apex, will be measured using a scale (Marcela *et al*., 2014).
5	**Vigour index (VI) (Length basis and Dry wt. basis)**	Vigour index will be calculated by multiplying the percentage of seed germination by the total length of the seedling (Abdul-Baki and Anderson,1973).
6	**Salt tolerance index (STI)**	Root length and shoot length will be measured at 10 DAS and Salt tolerance index (STI) will be calculated using formula given by Rahman *et al*. (2008).
Experiment 2: Effects of foliar application of Zinc sulphate and Zinc nanoparticles under saline water stress in pearl millet. (Pot study)		
1	**No. of varieties**	Two varieties from experiment 1 1. Most salt tolerant variety 2. Most salt sensitive variety

		(Based on experiment 1 results, the best performing variety will be taken as salt tolerant variety and poorest performing variety will be taken as salt sensitive variety.)	
2	**Details of salt application**	Appropriate salt concentration from experiment 1 is taken to irrigated pearl millet tolerant and susceptible variety under pot culture.	
3	**Synthesis and characterization of ZnO-NPs**	**Synthesis of zinc nanoparticles** • Chemical synthesis of zinc nanoparticles: Chemical synthesis of Zinc nanoparticles will be done by appropriate method at the time of research work. **Characterization of zinc nanoparticles** a. Qualitative analysis: UV-vis spectrophotometry (Jayachandran *et al*., 2021) b. Quantitative analysis: 1. FTIR (Abel *et al*., 2021) 2. SEM (Shubham *et al*., 2020) 3. EDX (Mofid *et al*., 2020) 4. Particle size analyzer (Naseer *et al*., 2020) 5. Zeta potential (Chaudhari and Malodia, 2017)	
4	**Treatments combination (Two foliar spray of Zinc sulphate and Zinc nanoparticle 1st At boot leaf stage and 2nd At 15 DAS after first spray)** **V_1 = Most salt tolerant variety** **V_2 = Most salt sensitive variety**	V_1T_1:	Salt tolerant variety (as absolute control)
		V_1T_2:	Salt tolerant variety + Saline water irrigation
		V_1T_3:	Salt tolerant variety + Saline water irrigation + Water spray
		V_1T_4:	Salt tolerant variety + Saline water irrigation + 500 ppm Zinc nanoparticle spray (Sumersinh *et al*., 2024)
		V_1T_5	Salt tolerant variety + Saline water irrigation + 0.5% Zinc sulphate (Girish *et al*., 2023)
		V_2T_6:	Salt sensitive variety (as absolute control)

		V_2T_7:	Salt sensitive variety + Saline water irrigation
		V_2T_8:	Salt sensitive variety + Saline water irrigation + Water spray
		V_2T_9:	Salt sensitive variety + Saline water irrigation + 500 ppm Zinc nanoparticle spray (Sumersinh *et al*., 2024)
		V_2T_{10}	Salt sensitive variety + Saline water irrigation + 0.5% Zinc sulphate (Girish *et al*., 2023)
		(Note: 1. In all the treatments, first two irrigations will be given with normal water and after germination saline water irrigation will be started. 2.The saline water concentration will be decided from experiment 1 based on performance of different varieties.)	
5	**Pot capacity**	15 kg soil/pot	
6	**Seed rate**	10 plant/pots, 5 plants will be maintained for sampling.	
7	**Recommended fertilizer dose**	As per RDF 80:40:00 N:P:K Kg/ha	
8	**Design**	FCRD (Factorial Complete Randomized Design) 1st Factor: Variety -2 2nd Factor: 10 treatments	
9	**No. of replications**	Three	
10	**Sampling**	Leaf sample of pearl millet will be collected at 10 Days after second spray for physiological, biochemical and antioxidant enzymatic activities analysis.	
11.	**Observations to be recorded**		
11A	**Physiological parameters**		

1	**Relative Water Content (RWC)**	Fresh weight, turgid weight and dry weight will be carried out for relative water content (Weatherly, 1962).
2	**Membrane Stability Index (MSI)**	The procedure for membrane stability index will be given by Sairam *et al*. (2005).
11B	**Biochemical parameters**	
1	**Chlorophyll content**	Chlorophyll a, Chlorophyll b and Total Chlorophyll content will be determined from pearl millet leaves by 80% acetone method (Arnon, 1949).
2	**Total Phenol**	Total phenol content will be determined from pearl millet using method by folin phenol method (Bray and Thorpe, 1954).
3	**Total soluble protein**	The total soluble protein content will be determined from pearl millet leaves using method by Folin-Lowry (Lowry *et al.*,1951).
4	**Total soluble sugars**	The total soluble sugar content will be estimated using the colorimetric method, as described by (Dubois *et al*.,1956).
5	**Free proline**	The free proline content will be determined spectrophotometrically using the ninhydrin method (Bates *et al*., 1973).
6	**Glycine betaine**	Glycine betaine will be determined from fresh leaves as per the method of Hendawey (2015).
7	**Free amino acids**	Free amino acid content will be analyzed by using the colorimetric method (Lee and Takahashi,1966)
8	**Lipid peroxidation**	Lipid peroxidase formation will be studies by Thiobarbituric acid (TBA) colour reaction from the control as well as from the salt stressed seed of pear millet (Heath and Packer, 1968).
9	**Protein profiling**	SDS PAGE and NATIVE PAGE method will be used as suggested by Singh *et al*., (2013).

10	**Metabolome profiling**	Untargeted metabolomics study will be perform using GC-MS. Metabolites is extracted described by Lisec *et al*. (2006) with minor modifications.
11C	**Antioxidant enzymes**	
1	**Superoxide Dismutase (SOD)**	About 3ml of buffer solution (0.1 M Potassium phosphate Ph- 6.8) containing 13mM methionine, 75µM NBT, 2µM Riboflavin, 0.1mM EDTA and appropriate aliquot of sample is used for SOD activity and absorbance will be recorded at 560nm (Dhinsda *et al*., 1981).
2	**Catalase**	About 3ml of buffer solution (0.1 M Potassium phosphate Ph- 6.5) is used. Appropriate aliquot was use for catalase activity and absorbance will be recorded at 240nm (Aebi, 1984).
3	**Peroxidase (POX)**	About 3ml of buffer solution (0.1 M Potassium phosphate Ph- 7.0) with 10Mm H_2O_2, 0.1mM EDTA, 10mM guaicol and appropriate aliquot of sample is use for guaicol peroxidase activity and absorbance will be recorded at 470 nm (Dias and Costa, 1983).
11D	**Yield and Yield attributes**	
1	**Days to 50% flowering**	Yield components will be recorded for each treatment at the time of harvest. (Kobraee and Shamsi, 2011).
2	**Plant height in cm**	
3	**Grain yield Kg/plant**	
4	**Ear yield Kg/plant**	
5	**Fodder yield Kg/plant**	
6	**No. of tillers/plant**	
7	**Test weight (1000 grain weight) in gm**	
8	**Threshing %**	

14 Statistical Analysis

Statistical analysis of the data will be conducted based on a Factorial Completely Randomized Design. The analysis of variance will be computed according to the standard methods described by Panse and Sukhatme (1985).

The data obtained from metabolomics analyses will be analysed using MetaboAnalyst software (Xia *et al*., 2009).

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